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(54) Title: METHOD FOR PROTECTION OF LUMBER AGAINST SAPSTAIN

(57) Abstract

The present invention consists of a method of protecting wood or wood products against unwanted sapstain by treating said wood with one or more biological control microorganisms selected from the genus *Gliocladium*, (fungi: Hyphomycetes), that either prevents the growth of undesirable sapstaining organisms, or prevents the formation of discoloration by these organisms.

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METHOD FOR PROTECTION OF LUMBER AGAINST SAPSTAIN

BACKGROUND OF THE INVENTION

Sapstain of unseasoned lumber is a cosmetic defect that is considered objectionable by many buyers. These discolorations, caused by a variety of microfungi, are a serious problem on lumber stored in lumber yards after sawing, but prior to planing and chemical treatment, and also on untreated lumber that is exported abroad. For the Canadian forest products industry, hem-fir products, spruce-pine-fir or white pine products are particularly prone to sapstain.

Several phenomena combine to create the discolorations. Sapstaining fungi generally discolour the wood brown, grey or black. The stain is caused by the pigmented fungal hyphae that accumulate in the cells of the sapwood, particularly in the rays. There is also evidence that dense accumulations of unpigmented hyphae in the wood tissue can cause similar discolorations. Some microfungi discolour wood by the production of coloured spores or sporulating structures. In addition, some species discolour wood red, purple, green or yellow by producing extracellular pigments that diffuse into the wood tissues.

Sapstaining fungi are primary colonizers of wood that subsist mainly on soluble nutrients. Although they cause little structural damage, they are perceived as forerunners of decay fungi by many consumers, and thus the objection to sapstain discoloration may have a more practical basis than just aesthetics.

Many different chemicals have been used to control sapstain. In Canada, the most widely used chemical formulations incorporate the chemicals, 2(thiocyanomethyl) benzothiazole, copper-8-quinolinolate, borax or didecyl-dimethyl ammonium chloride. The Canadian lumber industry has stated its intention of eliminating the use of toxic chemicals for sapstain control.

Biological control is a relatively new concept in forest products. In biological control, a "harmless organism", in this case one that does not decay or

discolour wood, is deliberately added to a product in order to prevent, retard or stop the growth of undesirable organisms. The most widely used biological control product is the bacterium Bacillus thuringiensis, better known as BT, which is used to control spruce budworm in Ontario and parts of Quebec. The bacterium produces a toxic crystal that is eaten by the budworm as it eats the leaves, eventually killing the pest. Approximately half a dozen biological control systems have been marketed for agricultural use, for example DeVine™, a fungal control of parasitic vines in citrus orchards. Below, some examples of biological control related to wood products pathology are reviewed.

The best known example of biological control in forest products relates to the control of decay in wooden transmission poles by the injection into the wood tissue of a dart containing spores or mycelium of so-called "immunising commensals", as described by J. Ricard in Canadian patents nos, 963387 and 1106201. Ricard claims a wide range of applications for his invention, but all claims of said invention relate to the possibility of inoculating biological control agents into wood tissue, and none of the claims relate to the prevention of sapstain.

Several research teams around the world have published results of screening programs for biological control organisms for the prevention of wood decay. The concept of Ricard, using mixtures of Trichoderma spp. and Scytalidium sp. to control decay in transmission poles, has been investigated by other research teams in the United Kingdom, the United States and the Federal Republic of Germany. Antifungal metabolites of Scytalidium sp. have been isolated and chemically characterised, along with antifungal metabolites from Hyalodendron sp. and Cryptosporiopsis sp., and these metabolites have been applied to wood in an attempt to prevent decay[1].

Another application of biological control organisms is to inhibit decay in round wood in storage.

Shields[2] reported that decay by Bjerkandera adusta, Coriolus hirsutus and C. versicolor was inhibited in wood blocks precolonized with Trichoderma harzianum or an unidentified strain (now known to be Scytalidium lignicola). The strain of T. harzianum was later used in a field test on birch bolts[3] where a conidial suspension was sprayed onto freshly cut ends of birch bolts. After a two week precolonization period, Bjerkandera adusta was inoculated onto the bolts. After six months, very little B. adusta was re-isolated from the bolts.

Stilwell[4] isolated a strain of Cryptosporiopsis sp. from yellow birch that inhibited the growth of 31 decay fungi in agar interactions. Decay of blocks by Fomes fomentarius was inhibited in precolonization experiments. In a field test, decay was reduced in peeled birch logs inoculated with a water suspension of Cryptosporiopsis sp., but no significant difference was noted in unpeeled logs. Culture filtrates of Cryptosporiopsis also inhibited growth of F. fomentarius. The antibiotic metabolite was purified, characterized and given the name cryptosporiopsin.[5]

Decay of wood chips during storage was also considered as a possible target for antagonistic micro-organisms. Bergman and Nilsson[6] tested several mould fungi isolated from wood chips for their ability to inhibit chip decay in laboratory experiments, and found that most decay fungi were inhibited. Gliocladium viride, a mycoparasite frequently isolated from chips, was tested on spruce chips in the field, and inhibited decay at temperatures less than 30°C, but failed at higher temperatures.

Conifer chips inoculated with an antibiotic-producing Cryptosporiopsis sp. and stored outdoors for 12-15 months yielded an improved quality of pulp although decay was not completely inhibited.[7] The results of trials using the antibiotic as a chemical preservative, and of a proposed field test, have not been published.

Bacteria were also tested as biological control agents in chip piles. Some bacteria isolated from hardwood

chips were inhibitory to selected decay fungi in agar interactions, but the antagonism was only effective on wood when the bacteria were inoculated onto the wood several weeks before the decay fungi.[8] The results of a planned field trial were not published.

The possibility of controlling sapstain by using antagonistic organisms has also received some attention. The early work of Stilwell and his colleagues[9] demonstrated the antagonism of some microorganisms towards some sapstain fungi. Stranks[10] found that 0.25% and 0.50% solutions of the antibiotic hyalodendrin, applied to white pine blocks by dipping, were effective at preventing sapstain by Graphium sp., while cryptosporiopsin was ineffective. Seifert et al[11] screened a variety of microfungi for their abilities to prevent sapstain precolonization experiments, and identified Nectria cinnabarina, Gliocladium roseum, Trichoderma spp. and Tympanis sp. as promising candidates.

Russian workers[12] have demonstrated in vitro inhibition of sapstain fungi by unidentified bacteria, but have not demonstrated efficiency on wood. Bernier and colleagues[13] showed that an isolate of Bacillus subtilus prevented sapstain when wooden blocks dipped into a cell suspension were placed on agar plates inoculated with sap-staining fungi, but subsequent work at Forintek Canada Corp. in Ottawa, Canada with the same culture showed that it did not inhibit sapstain when the wood was not placed on agar. The bacterium colonized wood very poorly and this prevented effective biological control. Benko[14] has recently screened many bacteria for antagonism towards sapstain fungi in agar interactions, and has selected some strains of Pseudomonas for further study.

Some innovative approaches towards biocontrol of sapstain have also been tried. Johnson[15] studied polyoxin, an antibiotic that inhibits the synthesis of chitin, a major component of fungal cell walls. The eight sapstain and mould species tested were sensitive to this compound but at concentrations too high to be economical on a

commercial scale. Benko[16] demonstrated that crude culture extracts of some antibiotic producing mycorrhizal fungi prevented growth of several sapstaining fungi on blocks of pine.

FIELD OF THE INVENTION

The present invention consists of a method of protecting unseasoned softwood lumber against unwanted sapstain by inoculation of said lumber with the unique biological control microorganisms from the genus Gliocladium, (fungi: Hyphomycetes), that either prevents the growth of undesirable sapstaining organisms, or prevents the formation of discolouration by these organisms.

The biological control organism is a fungus that does not itself decay or discolour the wood to any objectionable extent, and comprises one or more of the following strains: Gliocladium aureum (Forintek Culture Collection, FTK 784A), Gliocladium roseum (FTK 321A, 321M), Gliocladium solani (FTK 810A), Gliocladium viride (FTK 623E), Gliocladium virens (FTK 258C, FTK 258D).

SUMMARY OF THE INVENTION

The present invention relates to a method for the protection of wood or wood products against unwanted discoloration caused by sapstain fungi. It is a feature of the present invention to provide a method of controlling sapstain in wood and wood products comprising treating the wood or wood product with an inoculum comprising one or more fungi of the genus Gliocladium. The inoculum is of sufficient concentration and vigour to allow rapid colonization of the wood tissue by the inoculated biological control fungus. The actively growing and metabolizing biological control fungus does not itself damage or discolour the wood, but, likely through antibiotic facilities or mycoparasitism, protects against discoloration of the wood by undesirable organisms already present in the wood tissue, or that may be introduced to the wood tissues during handling of the lumber. Because the biological control fungus must subsist only on nonstructural wood

carbohydrates, the duration of control is of necessity finite, and can be expected to become less effective as easily assimilable nutrients are depleted, perhaps up to one year after inoculation of the wood with the biological control agent. The wood or wood product is preferably unseasoned softwood lumber such as conifer wood.

The invention also relates to a wood or wood product treated with a Gliocladium sp. Accordingly, it is another feature of the present invention to provide a wood or wood product treated with a fungus of the genus Gliocladium that is essentially free of sapstain as the result of the activity of said fungus.

The invention is intended for use primarily in situations where sapstain is prevalent, but for which chemical protection is impractical or impossible. Suggested applications include the protection of freshly sawn timber during seasoning, prior to planing and subsequent chemical treatment, protection of export lumber where chemical treatment, or specific chemical treatments, are forbidden by the importing countries, or treatment of wood chips during storage.

The genus Gliocladium is a biologically diverse group of moulds (Hyphomycetes) that includes species that are parasites of other fungi (mycoparasites), parasites of slime moulds, parasites of plant roots, and species that grow in soil and on wood. Gliocladium roseum is commonly isolated from soil in many parts of the world and is one of the most aggressive mycoparasites known. All the tested isolates are effective biological control agents. The isolates that we have employed are identified below. All of these were collected independently from each other as follows:

FTK 784A: Gliocladium aureum Rader, isolated by W.E. Rader from stored root of Daucus carota (carrot), received from H. Bruckner, (Germany) (Centraalbureau voor schimmelcultures 226.48, American Type Culture Collection 10406).

FTK 623E: Gliocladium viride Matr., isolated by M. Hawara from hardwood chip, Thurso, Quebec, May 30, 1988, identified by K.A. Seifert.

FTK 321A: Gliocladium roseum Bainier, isolated by C.K.J. Wang from soil debris, Natural Bridge, N.Y. July 11, 1980.

FTK 321M: Gliocladium roseum Bainier, isolated from yellow poplar stump, West Virginia, 1953/HL Barnett 914 (University of Alberta Microfungus and Herbarium 419).

FTK 810A: Gliocladium solani (Harting) Petch, isolated by T. Benedek. identified by W. Gams, (Centraal-bureau voor schimmelcultures 187.29).

FTK 258C: Gliocladium virens. Miller et al., from W.H. Weston T-1 (American Type Culture Collection 9645).

FTK 258D: Gliocladium virens. Miller et al. from Dr. S. Gyorgy, Budapest, Hungary, identified by J. Bisset (All-Union Collection of Non-Pathogenic Organisms, Institute of Microbiology, USSR Academy of Sciences - 1117).

Cultures of the isolates referred to above are available upon request made to Forintek Culture Collection of Forintek Canada Corp., 800 Montreal Road, Ottawa, Ontario, Canada, K1G 3Z5. Please note that any culture described in the specification that is available from the Forintek Culture Collection possesses a number that is preceded by the letters, FTK.

DETAILED DESCRIPTION OF THE INVENTION

The examples below describe the effectiveness of Gliocladium spp. in preventing or inhibiting sapstain or sapstain fungi, and demonstrate that Gliocladium spp. does not itself damage wood.

EXAMPLE 1

An inoculum of the biological control agent is prepared by removing plugs of agar from stock cultures and growing them on agar in petri dishes. The growth medium

employed is typically Difco™ malt extract with 2% agar (2% MA) for the Gliocladium spp. and for the sapstaining fungi.

Stock cultures are maintained at 4°C on agar media containing 2% Difco™ malt extract as a nutrient source. All other incubations described in this example are at 27°C, 75% relative humidity, in the dark.

The fungi used as biological control agents in this example are selected from the following strains maintained in the Forintek culture collection of wood-inhabiting fungi: Gliocladium roseum 321M, Gliocladium aureum 784A, Gliocladium solani 810A. Sapstaining fungi employed are: Ophiostoma piceae FTK 3871, O. piliferum (Fr.) H. & P. Sydow FTK 55F, FTK 55H and Ophiostoma sp. FTK C28.

After 1-3 weeks, a plug from the colony of the biological control agent is placed on one side of 2% MA in a 9 cm petri dish, and a plug from the colony of the sapstaining fungus is placed on the opposite side of the petri dish, such that the two fungi will grow together near the centre of the plate. Each possible combination of biological control agent and sapstaining fungus is set up. The plates are examined periodically and the interactions between the organisms are observed.

In all cases, the biological control agents inhibit the growth of the sapstaining fungus when the two colonies make contact. None of the Gliocladium isolates inhibit the sapstaining fungi before contact, suggesting that diffusible antifungal metabolites are not produced in this experimental design.

EXAMPLE 2

Inocula of the biological control fungi are prepared by transferring plugs of stock cultures onto Difco™ potato dextrose agar in 6 cm petri dishes as in Example 1. Stock culture maintenance and incubation conditions are as in Example 1.

The biological control agents are selected from the following strains: Gliocladium roseum 321A, 321M,

Gliocladium viride 623E, Gliocladium aureum 784A, and Gliocladium solani 810A, and Gliocladium virens 258C, 258D.

The wood blocks used in this example are Jack Pine sapwood 3 cm long and 1 cm x 0.5 cm in cross section. These are sterilized by gamma irradiation and placed in glass petri dishes, eight blocks per dish, upon w-shaped glass bars fashioned from 3 mm glass tubing, that rest upon 2 sheets of filter paper in which 5 mL sterile distilled water has been absorbed.

A spore suspension from 1-3 week old agar cultures is prepared. The colonies from G. roseum 321A and 321M, G. aureum 784A, and G. virens 258D are transferred into a sterile Waring™ blender and homogenized for 30 seconds in 75 mL sterile distilled water. Spore suspensions of G. viride 623E, G. solani 810A and G. virens 258C are prepared by flooding the agar plates with 6 mL of sterile distilled water and liberating the spores with an L shaped glass rod.

The spore suspension of each Gliocladium strain is squirted onto the surface of 64 blocks, (8 per petri dish) using a sterile syringe such that the entire length of the block, though not necessarily the entire width, receives some liquid.

The blocks are then incubated for 1 week. Staining fungus inocula are grown in 6 cm petri dishes on appropriate agar media for 1-2 weeks. Spore suspensions are prepared in the same way as described for the biological control strains above. The sapstaining fungi employed are: Ophiostoma piceae FTK 3871, O. piliferum FTK 55F, FTK 55H and Ophiostoma sp. FTK C28, plus a "soup" mixture of Cephaloascus fragrans FTK 3071, Ophiostoma piliferum FTK 55H, Black Yeast FTK 86-010-1-1-1, Aureobasidium pullulans FTK 132Q, Leptodontidium elatius FTK 268A, Cladosporium cladosporioides FTK 273D, Ophiostoma populinum FTK 671A, Ophiostoma perfectum FTK 703A, Phialophora botulispora FTK 707A, Leptographium sp. FTK 2A2,

Phoma sp. FTK 86-8-3-2-1, Alternaria alternata FTK 2G and FTK 2H.

The sapstaining fungi spore suspensions are then inoculated onto the surface of the wood blocks in a manner identical to that used for the biological control fungi. Each sapstaining fungi spore suspension is inoculated onto 2 groups of 8 blocks for each Gliocladium strain.

The wood blocks are incubated a further four weeks. The surface and interior of the wood blocks thus treated are free from discolorations caused by the sapstaining fungi, while control blocks inoculated at the same time with only sapstaining fungi become darkly discoloured after only 1-2 weeks. The results of this experiment are illustrated in Table 1.

EXAMPLE 3

In this example, the ability of the isolate Gliocladium roseum 321A to cause weight loss in Jack Pine blocks is tested. The standard ASTM soil block test (D1413-76) is used. In this method, 200 g of a 3:1 soil-sand mixture are placed in a 500 mL glass jar with 60 mL distilled water. A feeder strip made of red pine sapwood, 41 x 29 x 3.0 mm, is placed on the surface of the soil. The jars are sterilized for one hour, cooled, then re-sterilized for a second hour. The lids are then replaced with sterile culture lids with a microbiological filter with a 0.2 µm pore size fitted over a 5 mm hole in each lid. The soil is inoculated with an agar plug from a growing colony of Gliocladium roseum 321A and incubated for 3 weeks. Then, two sterilized 19 mm cubes of Jack Pine sapwood of known dry weight are added to each jar. After 12 weeks incubation, the blocks are dried and reweighed.

The weight loss caused by Gliocladium roseum 321A was 2%, and was not significantly different than the weight loss in the blank control. A typical decay fungus, Poria carbonica FTK 120AM, incubated under the same conditions, caused a weight loss of 33%.

EXAMPLE 4

In this example, the ability of Gliocladium roseum 321A to cause strength loss in wood is determined. Jack Pine wood beams are incubated in a modified soil block test and the impact bending strength is measured using the Toughness Testing (ISOD) machine, as detailed below.

The ISOD impact bending machine measures the force required to break a span of wood. A weight attached to a pendulum is released using a foot pedal, which pulls a chain attached to a vertical metal bar. The bar then is pulled into the sample (= impact), and the wood is broken. The force required to break the sample is determined by converting the value recorded by the pendulum of the machine (in degrees and minutes) to inch-pounds. The ISOD machine was modified for the smaller wood beams by reducing the weight on the pendulum and modifying the sample holder.

The soil block test was modified from ASTM stand and D-1413 to allow for the different block sizes. Rather than using glass jars, 1 L Nalgene^{TM} polypropylene jars are used. Each jar contains 400 g of a 3:1 soil:sand mixture and 120 mL of distilled water. Three red pine feeder strips, 4.5 x 2.5 x 0.5 cm, are placed side by side on the surface of the soil. The jars are autoclaved for one hour, cooled overnight, then autoclaved again the next day for one hour. The jars are cooled in a biological safety hood, and the lids replaced with culture lids. The culture lids are modified lids with a central hole, 5/16 of an inch in diameter, covered on the inner surface with a Gelman^{TM} filter to allow air exchange.

The wood beams, 9.0 x 0.75 x 0.75 cm, are prepared from green Jack Pine (Pinus banksiana) sapwood. The beams are sorted into sets with more or less the same number of growth rings. Only beams with the grain more or less parallel to the long axis are selected. The beams are sterilized by gamma radiation and frozen until use.

The inoculum for Gliocladium roseum 321A is a spore suspension in water made from a 1-2 week old 2% MA

culture. The spore suspension is inoculated onto the wooden beams, and the beams are preincubated for 1 week in a 1 L Nalgene polypropylene jar. At the bottom of the jar is filter paper moistened with distilled water, then alternating layers of glass rods and wood beams. All beams used in the experiment are from a matched set. After the preincubation period, five test beams are aseptically added to each soil jar. For controls, uninoculated test beams are aseptically added to jars. Five jars are set up for each treatment, for a total of 25 beams per treatment. All incubations are at 27°C.

After four weeks incubation, the beams are removed from the jars and placed on screen racks. The samples are then placed at a constant temperature and humidity and allowed to equilibrate for 7 days. The force required to break each beam is then measured with the ISOD toughness testing machine. The values are converted using the equation:

Toughness (inch-pounds) = pendulum weight x ($\cos A^2 - \cos A^1$) where A^1 is the initial angle, and A^2 is the angle of the pendulum at failure.

The readings given by the machine when no specimen was present are converted using the same equation, and this value is subtracted from the converted test values to give a corrected value.

Wood specimens colonized with G. roseum require 12.76 ± 0.32 inch-pounds to break. The control blocks, with no added fungus, require 11.93 ± 0.44 inch-pounds to break while the decay control blocks required 8.87 ± 0.45 inch-pounds. Statistical analysis revealed that test blocks incubated with G. roseum 321A do not undergo significantly more strength loss than uninoculated Jack Pine test blocks under the conditions employed.

EXAMPLE 5

In this example, the ability of the candidate strain Gliocladium viride 623E to prevent decay by known decay fungi Gloeophyllum trabeum FTK 47D and Merulius

tremellosus FTK 52H is determined. Retained strength, a measure of a candidate's decay-prevention ability, is the ratio of impact bending strength of test beams incubated with both the candidate biocontrol fungus and the decay fungi to the impact bending strength of test beams incubated with the candidate biocontrol fungi alone. This figure is expressed as a percentage. The experimental set-up is the same as the set-up in Example 4 except that the blocks containing the biological control fungi were added to a Nalgene™ jar/incubator which had been inoculated one week earlier with a decay inoculum.

The decay inoculum utilized was prepared by blending 150 mL of sterile water and growing agar cultures of two 9 cm petri plates from each of the aforementioned decay organisms. Five drops of this inoculum were placed on Jack Pine feeder strips in each Nalgene jar/incubator. After an incubation period of one week, the beams colonized with the biological control fungus were added and allowed to incubate a further four weeks. The impact bending strength for each test beam was determined (as in Example 4) and the retained strengths calculated.

The test beams inoculated with Gliocladium viride 623E had a retained strength of 94%, compared to 87% for the control test beams which contained no biological control fungi. G. viride 623E, therefore, prevented a significant amount of decay from occurring in the test blocks.

EXAMPLE 6

This example examines the ability of the Gliocladium spp. to protect larger pieces of lumber from sapstaining organisms in a small scale trial under conditions closely related to those in field trials. The Gliocladium strain was grown on two 14 cm agar plates (2% MA see Example 1) and allowed to sporulate. A spore suspension of each strain was prepared by flooding the surface of the plates with 50 mL of sterile distilled water, pooling the suspensions and diluting them to 4 liters. The final spore concentration was measured using a haemocyto-

meter. This concentration ranged from 2×10^4 to 1×10^6 spores/mL. Freshly sawn 1" x 6" x 15" white pine lumber, 28 pieces per strain, was dipped in these spore suspensions and placed in plastic bins, 14 pieces per bin. Control bins containing lumber with no biocontrol organisms were also set up. Humidity was maintained in these bins by the addition of 1L of sterile distilled water. The bins were covered with tight fitting lids equipped with ventilation holes covered with 0.22 µL Gelman™ filters. The bins were incubated in a ventilated temperature monitored shed at ambient temperature. After 6 and 14 weeks, each piece of lumber was rated for the evidence of surface mold, decay and sapstain fungi. A visual rating system for the presence of the specified fungi is used. A piece of lumber is rated as acceptable if it contains less than 10% surface area covered with stain. The results of these trials are summarized in Table 2. A statistical analysis of this data (based on the analysis of the Chi-squared test of homogeneity of proportions) reveals that G. roseum 321A and 321M, G. solani 810A, and G. aureum 784A afford the lumber a significant protection from sapstain, mould and decay while wood treated with G. viride 623E was not significantly different than the control.

Table 1

Results summarizing the ability of the candidate biological control fungi to prevent sapstain on Jack Pine.

Biocontrol Fungus (FTK No.)	Sapstainer (FTK No.)	Number of blocks stained
Gliocladium roseum (321A)	Ophiostoma picea (3871)	0/8 , 0/8
	Ophiostoma sp. (C28)	0/8 , 0/8
	Aureobasidium pullulans (132Q)	0/8 , 0/8
	Alternaria alternata (2G)	0/8 , 0/8
Gliocladium aureum (784A)	soup (see Example 2)	0/8 , 0/8
	Ophiostoma piliferum (55H)	0/8 , 0/8
	Phialophora botulispora (707A)	0/8 , 0/8
	Black Yeast (86-010-1-1-1)	0/8 , 0/8
Gliocladium roseum (321M)	soup (see Example 2)	0/8 , 1/8
	Ophiostoma piliferum (55H)	0/8 , 0/8
	Phialophora botulispora (707A)	0/8 , 0/8
	Black Yeast (86-010-1-1-1)	0/8 , 0/8
Gliocladium solani (810A)	soup (see Example 2)	0/8 , 1/8
	Ophiostoma piliferum (55H)	0/8 , 0/8
	Alternaria alternata (2H)	0/8 , 0/8
	Black Yeast (86-010-1-1-1)	0/8 , 0/8
Gliocladium viride (623E)	soup (see Example 2)	0/4*
Gliocladium virens (258C)	soup (see Example 2)	0/8 , 0/8
(258D)	soup	0/8 , 0/8

* readings taken from wood chips

Table 2. Summary of data from small scale field trials detailing the number of acceptable pieces of lumber after 6 and 14 week incubation periods.

Treatment	Acceptable Pieces (%)	
	6 weeks	14 weeks
Control (no fungi)	71	32
Gliocladium aureum 784A	100	82
Gliocladium roseum 321A	79	71
Gliocladium roseum 321M	100	82
Gliocladium solani 810A	96	86
Gliocladium viride 623E	54	32

REFERENCES

1. Unligil, H.H. 1978. Decay resistance of wood treated with fungal antibiotics: cryptosporiopsin, hyalodendrin, and scytalidin. Wood. Sci. 11: 30-32.

2. Shields, J.K. 1966. Wood Pathology. Rept. For. Prod. Res. Branch, Dept. For. Can., April 1964-March 1965: 48-52.

3. Shields, J.K. 1968. Role of Trichoderma viride in reducing storage decay of birch logs. Bimonthly Res. Notes Dept. For. Can. 24: 9-10; Hulme, M.A. and J.K. Shields. 1972. Effect of primary fungal infection upon secondary colonization of birch bolts. Mat. und Org. 7: 177-188.

4. Stilwell, M.A. 1966. A growth inhibitor produced by Cryptosporiopsis sp., an imperfect fungus isolated from yellow birch, Betula alleghaniensis Britt. Can. J. Bot. 44: 249-267.

5. Strunz, G.M., A.S. Court, J. Komlossy and M.A. Stilwell. 1969. Structure of cryptosporiopsin: a new antibiotic substance produced by a species of Cryptosporiopsis. Can. J. Chem. 47: 2087-2094.

6. Bergman, O. and T. Nilsson. 1979. Outdoor chip storage-methods of reducing deterioration during OCS. pp. 245-271. In: Chip Quality Monograph. Edited by J.V. Hatton. Pulp and Paper Tech. Ser. no. 5.

7. Stilwell, M.A. and G.M. Strunz. 1967. Antibiotic produced by a fungus. Rept. CFS For. Res. Lab., Fredericton. N.B.

8. Lapetite, D. 1970. Etude sur bois de l'action antagoniste des bacteries vis-a-vis des champignons lignivores. Mat. und Org. 5: 229-237.

9. Supra 4; Supra 7; Stilwell, M.A., R.E. Wall and G.M. Strunz. 1973. Production, isolation and antifungal activity of scytalidin, metabolites of Scytalidium sp. Can. J. Microbiol. 19: 597-602; O.K. Strunz, G.M., M. Kakushima and M.A. Stilwell. 1973. Scytalidin: a new fungitoxic metabolite produced by a Scytalidium species. J. Chem. Soc. Perkin Trans. 1: 2280-2283.

10. Stranks, D. 1976. Scytalidin, hyalodendrin, cryptosporiopsis-- Antibiotics for prevention of blue stain in white pine sapwood. Wood. Sci. 9: 110-112.

11. Seifert, K.A., C. Breuil, M. Best, L. Rossignol and J.N. Saddler. Screening of microorganisms with the potential for biological control of sapstain on unseasoned lumber. Mat. und Org.: 23 BD Heft 2.

REFERENCES (cont'd)

12. Vasiliev, O.A. 1968. (On the question of using the antagonism of fungi and bacteria for the protection of wood). Mach. Trudy Leningr. Lesotekh Akad. 110: 28-33 (in Russian).

13. Bernier, R. Jr., M. Desrocher and L. Jurasek. 1986. Antagonistic effect between Bacillus subtilus and wood staining fungi. J. Inst. Wood. Sci. 10: 214-216.

14. Benko, R. 1988. Bacteria as possible organisms for biological control of blue stain. Int. Res. Group on Wood Preserv., Document No. IRG/WP/1339.

15. Johnson, B.R. 1986. Sensitivity of some wood stain and mold fungi to an inhibitor of chitin synthesis. For. Prod. J. 36(3): 54-56.

16. Benko, R. 1987. Antagonistic effect of some mycorrhizal fungi as biological control of sapstain. Int. Res. Group on Wood Preserv., Document No. IRG/WP/1314.

CLAIMS:

1. A method of controlling sapstain in wood and wood products comprising treating the wood or wood product with an inoculum comprising one or more fungi of the genus Gliocladium.

2. The method according to claim 1 wherein said fungi is selected from one of the following species of the genus Gliocladium: Gliocladium aureum, Gliocladium viride, Gliocladium roseum, Gliocladium solani, and Gliocladium virens.

3. The method according to claim 2 wherein the Gliocladium aureum is FTK 784A.

4. The method according to claim 2 wherein the Gliocladium viride is FTK 623E.

5. The method according to claim 2 wherein the Gliocladium roseum is FTK 321M.

6. The method according to claim 2 wherein the Gliocladium solani is FTK 810A.

7. The method according to claim 2 wherein the Gliocladium virens is selected from FTK 258C or FTK 258D.

8. The method according to claims 1, 2, 3, 4, 5, 6 or 7 wherein said wood or wood product is softwood or conifer lumber.

9. The method according to claims 1, 2, 3, 4, 5, 6 or 7 wherein said wood or wood product is wood chips.

10. The method according to claims 1, 2, 3, 4, 5, 6 or 7 wherein said wood or wood product is pine wood.

11. A wood or wood product treated with at least one fungus of the genus Gliocladium said product being essentially free of sapstain as the result of said treatment of said fungus.

12. A wood or wood product according to claim 11 wherein the fungus is selected from one of the following species of the genus Gliocladium: Gliocladium aureum, Gliocladium viride, Gliocladium roseum, Gliocladium solani, and Gliocladium virens.

13. A wood or wood product according to claim 12 wherein the Gliocladium aureum is FTK 784A.

14. A wood or wood product according to claim 12 wherein the Gliocladium viride is FTK 623E.

15. A wood or wood product according to claim 12 wherein the Gliocladium roseum is FTK 321M.

16. A wood or wood product according to claim 12 wherein the Gliocladium solani is FTK 810A.

17. A wood or wood product according to claim 12 wherein the Gliocladium virens is FTK 258C and 258D.

18. A wood or wood product according to claim 12, 13, 14, 15, 16, or 17 wherein said wood or wood product is softwood or conifer lumber.

19. A wood or wood product according to claim 12, 13, 14, 15, 16, or 17 wherein said wood or wood product is wood chips.

20. A wood or wood product according to claim 12, 13, 14, 15, 16, or 17 wherein said wood or wood product is pine wood.

21. A method of preventing weight loss in wood and wood products comprising treating the wood or wood product with an inoculum comprising one or more fungi of the genus Gliocladium.

22. A method according to claim 21 wherein said fungi is selected from one of the following species of the genus Gliocladium: Gliocladium aureum, Gliocladium viride, Gliocladium roseum, Gliocladium solani, and Gliocladium virens.

23. A method of preventing strength loss in wood and wood products comprising treating the wood or wood product with an inoculum comprising one or more fungi of the genus Gliocladium.

24. The method according to claim 23 wherein said fungi is selected from one of the following species of the genus Gliocladium: Gliocladium aureum, Gliocladium viride, Gliocladium roseum, Gliocladium solani, and Gliocladium virens.

25. A method of preventing decay in wood and wood products comprising treating the wood or wood product with an inoculum comprising one or more fungi of the genus Gliocladium.

26. A method of claim 25 wherein said fungi is selected from one of the following species of the genus Gliocladium: Gliocladium aureum, Gliocladium viride, Gliocladium roseum, Gliocladium solani, and Gliocladium virens.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 B27K3/34

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	B27K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [o]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	MATERIAL UND ORGANISMEN vol. 23, no. 2, 1988, BERLIN pages 81 - 96 K.A.SEIFERT ET AL. 'Screening of microorganisms with the potential for biological control of sapstain on unseasoned lumber' see the whole document --- -/--	1-24

[o] Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14 OCTOBER 1992	30.10.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	DALKAFOUKI A.

International Application No

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	BIOLOGICAL ABSTRACTS vol. 90 , 1990, Philadelphia, PA, US; abstract no. 115251, see abstract & CAN. J. BOT. vol. 68, no. 7, 1990, pages 1578 - 1588 B. T. LUCK ET AL. 'Immunological discrimination between a sap-staining fungus and a biological control fungus'	1-24
A	---	25-26
X	BIOLOGICAL ABSTRACTS vol. 87 , 1989, Philadelphia, PA, US; abstract no. 52457, see abstract & MATERIAL UND ORGANISMEN vol. 23, no. 3, 1988, BERLIN T.L. HIGHLEY ET AL. 'Antagonism of trichoderma-spp and gliocladium-virens against wood decay fungi'	25-26 1-24
A	---	
A	FR,A,2 138 915 (INCENTIVE AB) 5 January 1973	
A	---	
A	US,A,4 996 157 (V.L. SMITH ET AL.) 26 February 1991	
A	---	
A	FR,A,2 158 065 (RICARD) 8 June 1973	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. CA 9200299 SA 62123

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 14/10/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A-2138915	05-01-73	SE-B-	373527	10-02-75
		AU-A-	4248772	22-11-73
		CH-A-	548269	30-04-74
		DE-A-	2224795	07-12-72
US-A-4996157	26-02-91	None		
FR-A-2158065	08-06-73	CA-A-	963387	25-02-75
		DE-A-	1929314	17-12-70
		DE-A-	1965970	03-12-70
		FR-A,B	2044750	26-02-71
		GB-A-	1274291	17-05-72
		GB-A-	1274292	17-05-72
		SE-B-	373526	10-02-75
		US-A-	4678669	07-07-87